A NOTE UPON THE UTILIZATION OF XYLOSE AND XYLAN BY *AZOTOBACTER*.

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It was observed in the course of earlier experiments (Jensen and Swaby, 1941), that hemicellulose in the form of crude xylan could be used for nitrogen fixation by associated cultures of *Azotobacter* and certain other bacteria which produced organic acids from xylan as well as from cellulose. Other cellulose-decomposing organisms, such as *Cellvibrio* spp., fungi and actinomycetes, did not give rise to any significant nitrogen fixation, although they readily broke the xylan down to reducing compounds, probably xylose. Similar results were afterwards obtained with several fungi, isolated from dew-retted flax, these decomposing xylan vigorously but cellulose only feebly. The following amounts of nitrogen, as averages of duplicate cultures of 25 ml., were found after incubation for 20 days at 28–30°C. in a medium containing: xylan 2·0%, yeast extract 1·0%, K_2HPO_4 0·1%, $MgSO_4$ 0·05%, NaCl 0·02%, $FeCl_3$ 0·01%, Na_2MoO_4 0·001% and $CaCO_3$ 0·2%.

Inoculum.	Total N, mgm.
Sterile control (analysed before incubation)	0·75
Az. chroococcum + *Cladosporium herbarum*	0·68
„ „ + *Stachybotrys atra*	0·72
„ „ + *Alternaria tenuis*	0·75
„ „ + *Aspergillus niger*	0·80

This constant failure of *Azotobacter* to fix nitrogen in association with xylan-hydrolyzing organisms suggests inability to utilize xylose, the main hydrolysis product of natural xylan. Only few observations have been recorded on the utilization of this and other pentoses by *Azotobacter*. Stoklasa (1908) stated that xylose was readily consumed with a yield of about 7 mgm. fixed N per gm. of sugar, but since he also reported the formation of ethyl alcohol, lactic acid and elementary hydrogen in considerable quantities from glucose, it seems open to doubt whether his *Azotobacter*-cultures were pure. Löhnis and Pillai (1908) found that crude cultures of *Azotobacter* utilize xylose very well, fixing about 9·5 mgm. N per gm. sugar. Hoffmann and Hammer (1910) found that it represented an inferior source of carbon for pure cultures, whereas Mockeridge (1915) reported a fixation of 9 mgm. N per gm. of xylose consumed. Stapp and Ruschmann (cit. after Bucherer, 1933) found it unavailable; Bucherer confirmed this, but stated that *Aspergillus niger* elaborates assimilable compounds from xylose. Georgi and Ettinger (1941) found xylose readily assimilable, but their experiments, like Bucherer's, were qualitative only. All these data refer to *Az. chroococcum*; other species do not appear to have been tested. The conflicting evidence might in part be due to the use of media deficient in molybdenum, but it is also possible that different strains may vary in their behaviour towards xylose (cf. Smith, 1935).

In order to clear up this somewhat obscure point, twenty-five strains of *Azotobacter* were tested for their ability to grow in xylose-media. They included twenty isolates of *Az. chroococcum* and one of *Az. vinelandii* from Australian soils, in addition to four of American origin (two *Az. chroococcum*, one *Az. beijerinckii*, one *Az. vinelandii*). Some of the local isolates were obtained by direct plating on N-free dextrine-agar, and others after previous enrichment in N-free xylose-solution inoculated with soil; these enrichment-cultures produced typical *Azotobacter*-pellicles after 3 to 4 days at 28°C. (cf. Löhnis and Pillai, 1908). Growth was tested in a solution containing 1% xylose and mineral constituents as above, and also on plates of a corresponding agar medium. Two batches of xylose were tried. Plate cultures were incubated for 8 days and liquid

cultures for 14 to 18 days at 28–30°C. No visible growth was produced by any strain except the local isolate of *Az. vinelandii*, which showed a very scant growth on agar and a barely perceptible turbidity in solution. A quantitative experiment was therefore carried out with this strain; duplicate cultures in 20 ml. 2% xylose-solution were incubated for 35 days at 28–30°C., and the following average amounts of nitrogen were found:

Inoculum.	Total N, mgm.
Sterile control solution	0·07
Az. vinelandii	0·35

Az. vinelandii thus seems able to utilize the xylose to a slight extent, but in *Az. chroococcum* this is obviously not a common property. Assuming that the cultures were pure, the xylose-consuming strains examined by Mockeridge (1915) and Georgi and Ettinger (1941) must consequently have been of an exceptional character; the strong acid-formation from several sugars in Georgi and Ettinger's experiments also indicates a somewhat abnormal behaviour.

The nitrogen fixation resulting in mixed cultures would evidently depend on the ability of the accompanying organisms to transform the xylose into compounds available to *Azotobacter*, and in their turn to utilize the nitrogen fixed by the latter. According to Thaysen and Galloway (1930), xylose is fermented by many different groups of bacteria, and some of them, e.g., the lactic acid bacteria, yield products that are valuable sources of carbon for *Azotobacter*. The same is true of some typical anaerobes such as the butyric acid and butyl alcohol bacilli; these or similar organisms may be responsible for the good growth of *Azotobacter* in xylose-solution inoculated with soil. An actual example of such an associate that enables *Azotobacter* to utilize xylose is furnished by a cellulose-decomposing corynebacterium (*Cor. fimi*?) which was previously found able to feed *Azotobacter* from cellulose and xylan (Jensen and Swaby, 1941). Pure and associated cultures in 20-ml. portions containing 2% xylose, 0·5% yeast extract, and minerals as above, showed after incubation for 18 days at 28–30°C. the following contents of nitrogen (average of duplicates unless otherwise stated):

Inoculum.	Total N, mgm.	Gain, mgm.
Sterile control (triplicate)	0·47	
Corynebacterium fimi (single)	0·46	
Az. chroococcum	0·48	
„ „ + *Cor. fimi*	1·27	0·80
Az. vinelandii	0·62	(0·15)
„ „ + *Cor. fimi*	1·21	0·74

The fixation by *Az. vinelandii* alone is so slight as to be of questionable significance, but both species together with the corynebacterium produced good growth and a definite nitrogen fixation. Also a variety of *Bac. polymyxa*, isolated from an enrichment-culture in xylose-solution, supported growth of *Az. chroococcum* in soil-extract with 1% xylose, but several other xylose-decomposing bacteria isolated from similar sources failed to do so, and likewise *Rhizobium meliloti* and *Acetobacter suboxydans*; apparently their metabolic by-products are not acceptable to *Azotobacter*.

Another test was performed to see whether *Bac. polymyxa* was able to induce growth of *Azotobacter* in association with xylan-hydrolyzing organisms. Various combinations were grown in 20-ml. portions of dilute soil-extract with 2% xylan, mineral constituents as above, and per culture, 0·5 mgm. N as $(NH_4)_2SO_4$. After 40 days at 28°C. the following amounts of nitrogen were found:

Inoculum.	Total N, mgm.	Gain, mgm.
Sterile control	1·28	
Az. chroococcum + *Bac. polymyxa*	1·21	
„ „ + *Cellvibrio* sp.	1·06	
„ „ + „ + *Bac. polymyxa*	2·57	1·29
„ „ + *Trichoderma* sp.	1·28	
„ „ + „ + *Bac. polymyxa*	1·25	

The combination of *Azotobacter* with *Cellvibrio* and *Bac. polymyxa* has fixed an appreciable quantity of nitrogen; this was also the only case where a visible growth of *Azotobacter* was observed. The corresponding combination with *Trichoderma* failed to fix nitrogen, probably because the fungus only accumulates reducing sugars from the xylan when deprived of oxygen (sealed cultures). Thus when nitrogen is fixed under

natural conditions at the expense of xylan, the biological mechanism would seem to consist of an association between *Azotobacter* and organisms like *Cor. fimi* or *Cellvibrio* spp., in the latter case with the intervention of organisms like *Bac. polymyxa*; the place of the last organism might be taken by *Clostridium butyricum* which, by virtue of its own ability to fix nitrogen, might further increase the efficiency of the process and also lead to a gain of nitrogen under conditions unsuitable for *Azotobacter*.

Arabinose, the pentose that comes next to xylose in importance as a structure-element of hemicelluloses, is stated by Stoklasa (1908), Hoffmann and Hammer (1910), and Mockeridge (1915), to be a favourable nutrient for *Az. chroococcum*, but Bucherer (1933), as well as Stapp and Ruschmann quoted by him, found it unavailable. Among the organisms included in the present experiments, the American strain of *Az. beijerinckii* produced a scant, and the local isolate of *Az. vinelandii* a good growth in arabinose-solution, but *Az. chroococcum* did not grow. A quantitative experiment (duplicate cultures in 25 ml. N-free mineral solution with 2% arabinose) showed the following amounts of nitrogen after 15 days at 28–30°C.:

Inoculum.	Total N, mgm.
Sterile control solution	0·06
Az. chroococcum	0·06
Az. vinelandii	11·28

The amount of nitrogen fixed by *Az. vinelandii* corresponds to more than 22 mgm. per gm. of sugar; this is an efficiency probably as high as ever recorded. A relatively strong nitrogen fixation might thus result at the expense of arabans or other hemicelluloses containing arabinose, through direct association of *Az. vinelandii* and araban-hydrolyzing organisms.

Summary.

Xylose was not utilized by *Azotobacter chroococcum*, and only to a very small extent by *Az. vinelandii*. Nitrogen can be fixed through the intervention of other bacteria capable of transforming the xylose into compounds available to *Azotobacter*. Xylan may be utilized by *Azotobacter* in association with such organisms in addition to others that hydrolyze the xylan. Arabinose is utilized very well by *Az. vinelandii*, but not by *Az. chroococcum*.

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